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MICROSCOPICAL IMAGE FORMATION.

BY F. J. KEELEY.

This subject is of importance to every user of a microscope, as a correct understanding of the optical laws which govern the formation of the image would assist in determining to what extent the true structure and size of the object under examination is correctly represented by the appearances presented to the eye. We are confronted with two radically different theories of microscopical vision, which may be termed the diffraction and dioptric theories, although the former requires the presence of both diffracted and dioptric beams, while the latter deals principally with the effect of diffraction, but only such as arises in the instrument itself and not from the object under examination. As the exponents of each seem to take partisan views and fail to give adequate consideration to the opposing theory, it appeared to be worth while to make a careful study of both in as far as possible an unprejudiced manner, supplemented by experiments, with the view of endeavoring to learn whether they are wholly irreconcilable.

From 1771, when Benjamin Martin applied a low power achromatic objective to a microscope and explained that its superiority was largely due to the increased aperture available, until Tolles, a century later, realized the possibility and practicability of producing objectives with apertures in excess of the equivalent of 180° in air, the principal efforts of the opticians and their collaborators, the microscopists, was in the direction of increased aperture and improved chromatic corrections, and no serious doubts appear to have been raised regarding the dioptric theory, in the form in which it had been worked out for the telescope; but when the practical limit of aperture with available materials had been reached by all the leading opticians, more attention was paid to theoretical questions, and it began to be apparent that there were certain phenomena connected with image formation in the microscope which could not be satisfactorily explained by accepted theories. Abbe investigated this problem with characteristic acumen and evolved the theory which has since borne his name, and although immediately attacked and later somewhat modified by himself, although in a radical direction,

his theory has stood unrefuted up to the present time; but recently there has been a feeling aroused that, without further modification, it fails to fully account for all features of observed microscopical images.

The old dioptric theory has accordingly been re-expounded and amplified, and it is unnecessary to explain it in detail, as this has been most admirably and completely done in Wright's "Principles of Microscopy," published in 1906, which, while characterized by inaccuracies in connection with such matters as the measurement of focus and aperture, and indicating a lack of acquaintance with the diffraction theory, covers the ground from the dioptric point of view so thoroughly that I find but one point open to criticism, which is the employment of bright points and lines, equivalent to self-luminous objects, in explaining the formation of the diffraction patterns termed "antipoints." This is not a condition which is met in practical microscopy except with dark ground illumination, but every skilled microscopist knows that this form of illumination, although advantageous for many purposes, particularly for increasing the visibility of isolated points and lines, is never equal to direct light in developing true images of structure, the elements of which are not separated by distances exceeding their own diameters. The decreased resolution has recently been explained by Nelson, and, in addition to this, false appearances intermediate between true structural elements may be produced by the coincidence of diffracted rays, which would not be visible with direct light.

In the rare case of opaque particles in a transparent medium, such as is furnished by a rock section containing minute crystals of magnetite or pyrite, the phenomena observed at the margins of these crystals may be explained as just the reverse of those arising from a bright point image; but in the vast majority of all structures examined with the microscope there will be little difference in the illumination on opposite sides of a marginal line, possibly only a difference in color, and whether the margin be considered as imaged by slightly refracted rays or by slightly deflected diffracted rays, whether an image of the margin is produced at all or only an interference of "antipoints," it is unquestionable that in certain classes of objects at least, as my experiments have not covered the whole ground sufficiently to generalize, there is something visible corresponding to a limiting margin, which may be recognized and measured by a practiced eye, whether isolated when illuminated by a wide cone or flanked on both sides by diffraction bands with a narrow one. This fact had been impressed on me by

previously made experiments, for when I first realized the effect that the size of the illuminated aperture should have on the apparent size of an object, the theoretical basis for this conclusion seemed so sound that I started a series of measurements to establish a table of corrections for "antipoint" for future use.

Failing to get any consistent results, or even any variations beyond the probable limits of instrumental error, in the course of micrometry of various objects at odd times, I finally made the following measurements with higher powers, adopting as most suitable objects two well-stained human blood corpuscles, mounted in balsam, and so marked that they could be found at any time, not only because they represented a class of objects frequently subjected to measurement, but also for the reason that they furnished an absorption image that should be particularly susceptible to dioptric law. Full data are as follows:

1. April 18: Stand, Zentmayer Centennial. Objective, Zeiss 3 mm. Apochromat, 1.40 N. A. Condenser, Powell & Lealand Apochromat, 1.40 N. A., no diaphragm, focussed for greatest aplanatic aperture, about 1.20 N. A. Stage Micrometer, Rogers "A" Division, third one-thousandth inch, Zentmayer Filar Micrometer. Diameter of corpuscle No. 1, .00032050 inch; diameter of corpuscle No. 2, .00031745 inch.

2. April 18: Everything exactly as above, except that a diaphragm was inserted in the condenser, reducing the cone of illumination to .20 N. A. Diameter of corpuscle No. 1, .00031860 inch; diameter of corpuscle No. 2, .00031747 inch. Variation from first measurements, corpuscle No. 1, —.00000190 inch; corpuscle No. 2, +.00000002 inch.

3. April 19: Stand, Zentmayer Centennial. Objective, Bausch & Lomb one-eighth inch, .85 N. A. Condenser, Beck Achromatic, 1.00 N. A., diaphragmed to .20 N. A. Stage Micrometer, Rogers "A" Division, third one-thousandth inch, Zentmayer Filar Micrometer. Diameter of corpuscle No. 1, .00031890 inch; diameter of corpuscle No. 2, .00031665. Variation from first measurements, corpuscle No. 1, —.0000016 inch; corpuscle No. 2, —.00000080 inch.

4. April 19: Stand, Watson Van Heurck. Objective, Beck one-eighth .90 N. A. Condenser, Bausch & Lomb Abbe Achromatic 1.00 N. A. diaphragmed to .50 N. A. Stage Micrometer, Fasoldt "A" Division, second one-thousandth, Bausch & Lomb Filar Micrometer. Measurement of corpuscle No. 1, .00031609 inch. Variation from first measurement, —.00000441.

5. April 28: Stand, Zentmayer Centennial. Objective, Bausch & Lomb one-twelfth inch, W. I., 1.00 N. A. Condenser, Beck Achroma-

tic, 1.00 N. A. diaphragmed to .50 N. A. Stage Micrometer, Rogers "B" Division, tenth two-thousandth inch, Zentmayer Filar Micrometer. Diameter of corpuscle No. 1, .00031770 inch. Variation from first measurement, —.00000280 inch.

These measurements were made with the utmost care, the mean of ten readings of the filar micrometer being taken in each case, and between the readings both the object and lines of filar micrometer moved and the focus changed, so that each reading required independent adjustment in all respects. The actual value of the micrometer divisions was not determined until after all the readings on the corpuscles were made, to avoid any possibility of unconscious mental bias. Fractions of an inch are employed because my best micrometers, those with the sharpest defined lines, are so ruled. It is easy to calculate the measurements to microns if desired, but this was useless in present case where the measurements had no value save for comparison.

It will be noted that the first two measurements, with illuminating cones of 1.20 and .20 N. A. respectively, agree far within the limits of probable instrumental error. In fact the small variation on corpuscle No. 2 is entirely a chance result, and I have experienced such close results in but one or two other cases out of many hundreds of measurements. The measurements on the second evening, under still different conditions, strongly confirmed the previous results. The last two measurements, each of but one corpuscle, were made principally to test the accuracy of the stage micrometers used, and the greater variation shown, which in the fourth measurement approaches what I would consider the probable limit of instrumental error under the conditions, might be ascribed to the use of different stage micrometers. I doubt if this is entirely true, as all my micrometers have been so thoroughly and repeatedly compared and studied, that any division or divisions of any one of a half dozen or more might be used with little chance of serious error, the true value of each, as recorded in my notebook, being of course used in making the comparison, as well as the same marked position on the division, as all stage micrometers vary materially not only in different divisions but in different parts of same division. The best have a horizontal line on which all comparisons should be made. It is more likely that the comparatively large variation in the fourth measurement is mostly due to the employment of the Bausch & Lomb instead of the Zentmayer Filar Micrometer. While the former is very accurately made, it has ruled lines instead of spider webs, and therefore does not admit of such accurate definition

and placing of the lines, measurements being of course made between the interior edges of the lines, and the diameter of the latter allowed for in comparing with stage micrometer. In the fifth measurement, a slight error might occur from comparison with a different-sized division of stage micrometer, as owing to the variation in magnification over the field of ocular, even if errors of screw are immaterial, no filar micrometer is likely to give exactly double the reading on two equal divisions that it will on one of them. This difference, if only centre of field is employed, is too small to be serious, but in very accurate measurements it is always well to compare with divisions of stage micrometer as nearly as practicable corresponding to size of object measured.

From these data it will be evident that the unquestionable presence of "antipoint" phenomena need not be taken into consideration in connection with micrometry with white light, as a trained eye can select the same margins to measure under any ordinary conditions of illumination. This is not in the least contradictory to the theory, but demonstrates the possibility of overcoming a theoretical difficulty in actual practice. As anyone skilled at micrometry is likely to use a large cone whenever practicable, the only occasions when this question will be of importance will be when successive measurements must be made through covers of widely differing thickness. Owing to the impossibility of making any adjustment for this variation, it may be necessary to cut down the cone of illumination and depend on experience to select the margins to be measured.

In considering diffraction phenomena originating in the object, it will be well to first assume conditions under which the objective will be of greater aperture than the illuminating cone, and will therefore, when examined with ocular removed, exhibit a disk of light, the dioptric beam, surrounded by an unilluminated space. The insertion of an object in the focus of the objective will result in this dark space showing more or less light which may be both refracted and diffracted by the object. If the latter has a fine structure, periodically arranged, this light reaching the outer zone of the objective's aperture will be mostly of diffraction origin and will take the form of separated spectra. As is well known, the examination of such spectra will enable us to predicate in advance what will be the arrangement and distance of the structural elements visible, or if the objective is not of sufficient aperture to include at least the first order spectra, no image of the structure will be seen. Thus in arranging the illumination for the resolution of a difficult object—for instance, *Amphipleura pellucida* in dots, with a suitable objective—the easiest way is to pay little attention to

the image seen through the ocular, but to remove the latter after focussing, and modify the illumination until two spectra at right angles, as well as the dioptric beam, are visible in the back of the objective. Then on inserting the ocular the resolution should be apparent at once, or by making any necessary spherical corrections by means of adjustment collar or tube length; but as long as both spectra are not seen, it is utterly useless to endeavor to effect resolution by altering the adjustment. If after satisfactory resolution be secured one of the spectra be obscured by an intruding point, only lines at right angles to the other spectrum will remain visible.

Another well-known experiment is available with any objective capable of resolving *Pleurosigma angulatum* with a very small central cone, in which case the six characteristic diffraction spectra, free from refracted rays, may be seen at margin of objective. A cover glass, on which have been marked with India ink six dots that will cover the diffraction spectra, may now be placed behind the objective, and no trace of resolution will be visible; but if the cover glass be rotated 30° so as to bring the dots between the spectra, the resolution will be as good as before. We here have a case where radically different results are obtained without changing the character of the screen behind the objective, and any argument that it acts as a diffraction grating is invalid, because if this were the case it should so act and produce similar results in one position as well as the other. It is now evident that there is some connection between these diffracted beams and image formation in the microscope, and the question becomes whether the formation of the image is dependent on their presence, or whether they are merely accompanying phenomena which happen to appear concomitantly with conditions which would permit of similar image formation in their absence.

That they are actually image forming rays can be readily proved in several ways, the easiest being to throw the objective out of adjustment sufficiently that the rays from its outer zone, which will be the diffracted rays, are brought to a different focus from those of the dioptric beam in the centre, and the two images can thus be examined successively by a slight change in focus. A more convincing method is that of Rheinberg, in which the rays from outer zone are refracted out of the optic axis and the two images can be examined or photographed simultaneously. Still a third method will shortly be referred to in connection with the case of an objective illuminated by a solid cone of light of its own aperture, which must now be considered.

This is a condition we rarely meet in practical microscopy except

with low powers, as no wide-angled objectives have been made that are sufficiently well corrected to stand a full cone without breaking down, unless on objects such as deeply stained bacilli, where the image is formed principally by absorption and is practically a silhouette. There seems to be no reason why such images should not be regarded as dioptric, although there must be some diffracted rays from the margins which are undoubtedly utilized in the image.

When the back of the objective is entirely filled with light, the study of diffraction phenomena becomes difficult, as even with particularly suitable objects the diffraction beams are eclipsed by the brighter dioptric beam. As we open the iris of the condenser, however, it can be seen that the diffracted beams expand to the same extent as the dioptric beam, finally overlapping it and each other, until when the aperture of the objective is entirely filled with dioptric light it must unquestionably be similarly filled with diffracted rays. Unfortunately, there seems to be no way in which we can completely separate the beams derived from the two different sources, and the best expedient I could devise to obtain some idea of the conditions present consists in the use of a semicircular diaphragm in the condenser, so oriented that the left side of the back of objective is completely filled with light up to its margin, while the right side receives no direct rays whatever. Thus we have full cone conditions on one side, while the other will receive only refracted and diffracted rays, or if certain suitable objects are employed nothing but diffracted rays, whose behavior we can study separately.

For this purpose a binocular microscope should be employed with a specially short mounted objective, say a sixth of about .80 N. A., the back lens of which will come close to the Wenham prism. All the direct light from the fully illuminated left half of the objective will now pass up the right-hand tube of the microscope, while the diffracted beams from the right-hand half of objective will be reflected up the left tube. Assuming that *P. angulatum* is again the object, as it furnishes spectra singularly free from all indications of refracted light, and oriented longitudinally across the field in a right-and-left direction, on examining the back of objective, the previously dark space on the right will be found practically filled by three of the characteristic spectra of the object, and the other three will be present, although invisible, in the illuminated half, where they will occupy positions near the margin, as they can only be derived from central or nearly central dioptric rays. The diffracted beams in right half of objective, being derived from the dioptric beam which completely fills the left half,

will extend from close to the centre of objective to beyond its margin, and correspond in all essential respects to those present in a fully illuminated objective, as such widely diffracted beams must be derived from dioptric rays which pass through that portion of the objective's aperture diametrically opposite to them. It is true these beams would be expanded if the full aperture was illuminated, but the expansion would be principally outside the margin of objective and, as far as present experiment is concerned, be immaterial. The following results demonstrate that three such spectra as we are considering will produce an image identical with that resulting from the whole six, and the conditions may be summed up as follows:

Through the right-hand tube of the binocular, the image will be produced by a full dioptric beam, supplemented by diffracted beams corresponding to those resulting from a small central illuminating cone, while through the left-hand tube it is derived from diffracted beams alone, corresponding to those present with a full cone of illumination. On examination, it will be found that both images are fairly well defined, but that the resolution of the fine structure is noticeably sharper and more distinct in the diffraction image through the left tube.

It will also be noted that the diffraction image is blue in color, and before going further it will be well to fully understand how the colors of such images are to be accounted for. The diffracted beams seen at back of objective correspond to any other diffraction spectra and include light of such wave lengths as enter into their formation. The images resulting from their recombination will accordingly have the same color as the light supplied to the microscope, as modified by absorption in passing through the object, provided complete spectra are included within the aperture of the objective; but should part of the spectra be cut off at its margin, the diffraction images will correspond to color sensation produced on the retina by light included in that portion of the spectrum admitted, and will accordingly depend on two factors: the distance between the elements of the object and the aperture of the objective. Under the conditions outlined in present experiment it has already been noted that the diffraction image of *P. angulatum* is blue, which is due to the red ends of its spectra being cut off at margin of the objective's aperture. If *Navicula Lewisiana* or a coarse *N. rhomboides* be substituted only the violet end of the spectra will be admitted and the unresolved image be similarly colored. On the other hand, *Pl. formosum*, whose spectra will be completely admitted, will appear just as white in the diffraction as in the directly

illuminated image. Intermediate forms may appear green or even of a yellowish cast, but never red.

The agreement in color of the images seen through the left tube with what would be predicated from the examination of spectra admitted, is further evidence that they are due practically exclusively to diffracted rays, but still further experiment is required to demonstrate that they are free from refracted rays. The spectra here seen, and which have previously been considered, consist of an infinite number of overlapping images of the aperture in the substage diaphragm, each produced by light of a different wave length; hence only the extreme ends are practically pure colors, the middle portions of the spectra consisting of a jumble of colors sometimes producing the effect of white light; but if a narrow slit diaphragm be inserted in the condenser we can secure a spectrum that corresponds in sharpness to that from an ordinary spectroscope. This may be enlarged by the use of a low power objective in the draw tube, focussed on the back focal plane of the first objective as when using an Abbe apertometer. The microscope may now be used as a spectroscope, and by allowing the light supplied to it to pass through some coloring matter which has well-marked absorption bands, these will be visible in the spectra at back of objective. A solution of Eosin, which will be needed for a later experiment, will likewise answer here. It should be of such strength that when placed in a glass trough and examined with a spectroscope of low dispersion, it will show a black, well-defined band in the green adjoining the yellow, but allow the remainder of the spectrum to pass freely. On placing this trough in the path of the light used to illuminate the microscope, which should be an intense one, it will be noted that the absorption band in the spectrum derived from the diatom structure is perfectly black, furnishing a demonstration that it is practically free from refracted rays; for if it contained scattered refracted rays, as has been claimed, the absorption band would not appear black, but of same color as the light illuminating the object, which in this case is visually a bright red.

If we now examine the diffraction image produced under these conditions, taking the precaution to so adjust the slit diaphragm that the red end of diffraction spectrum is cut off by the margin of the objective's aperture, will find that it is just as blue with this visually red light as it was with white light, owing to the fact that Eosin transmits the blue rays freely; but the midrib of the diatom, and particularly any granular incrustation, such as may usually be found at places between the valve and cover-glass, will be tinted red, indicating that

refracted rays or complete diffracted beams enter into their image formation.

Three important questions have now been settled: Firstly, that the diffracted beams from certain structures are free from refracted rays; secondly, that sharp distinct images may result from such diffracted beams exclusively; thirdly, that where such beams are sufficiently separated from the dioptric beam to permit of their being eclipsed by a suitable screen, no image of the elements producing them will be visible.

Returning to the conditions of the previous experiment, we will substitute for the diatom a well-stained blood slide, preferably pathological, containing large eosinophilous leucocytes. As this is mounted in balsam, the corpuscles would be scarcely visible were they not stained, hence the image must be produced principally by absorption and little refraction can be expected. There must be some diffraction at the margins, but the diffracted beams thus produced will be slightly deflected, and for all practical purposes indistinguishable in their effect from refracted rays. After examining the image through right tube, and noting that practically nothing can be seen save by contrast of color, even the granules of the leucocytes being "drowned out" by the wide cone of illumination, the eye should be rested for several minutes in a dark place, to recover its sensitiveness not merely to faint images, but, what is particularly important in this case, to color sensation, which will have been impaired by the brilliant red color of the stained blood. When this has been done, the image through left tube may be inspected, and as anticipated it will be found exceedingly faint. Mere sketchy outlines of the corpuscles will be seen, of a reddish tint, but what will most impress the observer will be that the granular texture of the leucocytes will be quite distinctly imaged and of a bluish-gray tint. It is hardly necessary to state that this indicates diffraction origin of the image if the diffracted beams are deflected sufficiently to be partly cut off at margin of objective, as the distancing of the granules would indicate should be the case. It has already been shown why the visually red Eosin stain presents no serious difficulties in the way of a contrasting diffraction image.

In these experiments the position of the semicircular diaphragm might be reversed so as to give the diffracted beam the advantage of the direct tube, but in a good Wenham binocular it makes little difference which tube is used. I may be particularly fortunate in this respect, but among my own binoculars this is the case, and in at least two of them the difference in definition between the two images is not

greater than is commonly found between that of two objectives of identical construction from the same maker. Right here it may be well to mention two other fallacies regarding the Wenham binocular which seem likely to be perpetuated in microscopical literature, having even found a place in Spitta's recent valuable and generally accurate work on the microscope. One of these is, that the beam passing up the left tube produces a larger image (unless this is counteracted in the ocular) because it has to travel farther on account of the two reflections in the prism. As I many years ago explained, the path of the beam through the prism is but slightly over one and a half times the length of the path of direct beam alongside the prism. As this distance is in glass of at least 1.50 R. I. the ray emerges from the prism as if it had traveled only two-thirds as far in air, and the optical tube length is practically identical in both tubes, the difference not reaching a millimeter with an ordinary well proportioned prism. The other fallacy is that such binoculars are not suited for higher powers than about a half inch, while they really work well with objectives up to an eighth inch or higher, if the latter are mounted, as Wenham himself recommended, in short mounts, so as to avoid parallax between back lens and prism. If anyone doubts the immense advantage of stereoscopic effect with high powers, let him attempt with a monocular to demonstrate the anatomy, particularly the position of the unextruded proboscis, in a glycerine-mounted specimen of the motile condition of our too common pest, the San José scale, which I have never seen correctly figured, and then note the difference when a binocular is employed. The sculpturing on spores and capillitium of Myxomycetes, spores of ferns, etc., afford other good tests of its efficiency.

Returning to diffraction phenomena, I almost hesitate to refer to the two experiments by which Wright seems to think he has demolished the Abbe theory, as their fallacy is so self-evident that it must have been at once recognized by every microscopist having more than the most elementary acquaintance with the subject. The assumption, in the first experiment, that when the aperture of objective is fully illuminated there are no diffracted rays present, because they cannot be seen, is absurd, and needs no further consideration; but anyone desiring to know just what is the character of the diffracted beams resulting from any particular structure under the conditions specified, may secure the information by employing a pinhole diaphragm in a freely moving carrier connected with substage condenser. A circle should be drawn on a piece of paper to represent back of objective, and the diffracted beams visible plotted in with a red and blue pencil.

Diaphragm may then be moved slightly and the diffracted beams again recorded on same drawing. This can be repeated until the illumination from the pinhole has traversed every portion of the objective's aperture, or until the patience of the observer is exhausted, when, if the diaphragm be removed and the objective fully illuminated, it will be absolutely certain that all the diffracted beams represented on the composite sketch will still be present within the aperture of the objective. Twenty years ago Abbe published a paper dealing specially with fully illuminated apertures.

The other experiment described, which states that the image of an Abbe diffraction plate, illuminated through a narrow central aperture, and examined under a one-inch objective, becomes invisible when sunlight is used as the source of illumination, would be equally fallacious if true, as the glare of such light might prevent any image being seen, but its presence could be demonstrated by receiving it on a ground glass screen or by photographing it. But even the facts are here misstated. I did not doubt that the image might be obscured by the blinding effect of direct sunlight, and merely carried out the experiment to demonstrate that the image would appear on the screen of a camera, which from photomicrographic experience I knew would be the case; but I found not only that the lines were well defined on the ground glass of the camera, but also were equally distinct when examined directly through the microscope. Although Wright's description of the experiment had been followed in every detail, the conditions were likewise varied in every possible way that might lead to the results claimed. A shade prevented the sunlight from reaching any part of the microscope except the mirror, but in addition it was wrapped in several folds of a heavy focussing cloth, preventing the possible entrance of any light save the narrow central beam, which was modified in size first with the iris and then by a specially made pinhole diaphragm, much smaller than could be secured with the iris, or with the diaphragm accompanying the Abbe diffraction apparatus, recommended by Wright for the purpose. The disk of the sun was focussed sharply in the plane of the lines and also above and below; the condenser removed and sunlight merely restricted by the minute and larger diaphragms tried; eyepieces from two-inch to one-fifth inch employed; the fine one-inch objective at first used removed and replaced by the poorest one of this power in my collection, a cheap single system affair of low aperture; but it was useless to change the conditions, as under any of them the image remained just as sharp and distinct as it would have been with lamplight, and the only result

of an hour's experimenting was a tired eye and the conviction that it was absolutely impossible for anyone with eyesight good enough to use the microscope at all to perform this experiment with the results claimed. Nevertheless, devoid as it is of the slightest basis in either fact or deduction, it is offered as one-half of all the evidence necessary to refute so well considered a theory as that of Abbe. As it should succeed under the dioptric theory, it indicates the inadequacy of that theory alone to account for microscopical image formation.

One more experiment, of the many made, may be worth mentioning. For this will be required an objective that will stand full cone illumination, and a Nobert test plate or other series of rulings will supply the object. No objective of more than very low power and aperture will fully fill the specifications, which would require resolution with a full cone, equal to that with oblique light, but an old Spencer one-half inch of 70°, which approaches perfection more closely than any other objective I have seen, was found to answer the purpose. If illuminated with a cone completely filling its aperture, it will be found that the seventh band of the Nobert plate is well resolved, and if the aperture of the illumination be cut down as far as the iris will close, so the dioptric beam seen at back of objective will not exceed one-twenty-fifth of the diameter of the back lens, the third band, which is just twice as coarsely ruled as the seventh, will still be easily and distinctly resolved. Now the aperture of the illuminating cone has been cut to one-twenty-fifth, and by a strict interpretation of the dioptric theory the resolution should be impaired to a similar extent, but it is found to be one-half as great as with the full aperture. This will be answered by the statement that the image is now formed by refracted rays, outside the dioptric beam. Very well, if that is the case, then one-half of the aperture of the objective is sufficient to resolve the third band, and it can therefore do no harm if we contract the aperture just a little, say 10 per cent., by means of an iris back of it. It will be found, however, that as soon as this is done the lines completely disappear. In fact, just as soon as two minute diffracted beams, visible at margin of objective, are partially eclipsed resolution is at an end.

This experiment, dealing with the "mystery of the dark space" which led Abbe to evolve a new theory, is introduced not to demonstrate that the unilluminated portion of an objective's aperture assists in image formation, which every microscopist must already know, but to call attention to the fact that it does so in a definite manner. Under the explanation given by the advocates of the dioptric theory

alone, different results should be secured according to the character of the object. In the case of structure that could refract but little light the resolution would be cut down to 10 per cent. or less, owing to the large "antipoint," which would decrease in size and permit of greater resolution as the power of the object to refract rays increased, until in exceptional cases resolution practically equal to that with a full cone should be attainable. On the contrary it will always be found that, without regard to the character of the object, the resolution with central light will be just about one-half that with a full cone, or in other words, will correspond to the diffracted beams admitted by the objective.

In the absence of suitable rulings, the fact referred to may be demonstrated with diatoms or any other objects that may be at hand in sufficient variety, by noting the finest structure resolved under the two conditions of illumination, and then carefully counting the elements of the structure resolved in a given space. On a Moller 60 diatom test plate in styrax, the markings on all its forms having been accurately counted and recorded, it was found that the above objective with full cone, resolved *Grammatophora serpentina*, 48,000 per inch, and with central light reached its limit at the third *Navicula lyra*, 24,800 per inch, confirming the results from the rulings.

This objective, it may be mentioned, will resolve with oblique light, *Navicula Lewisiana*, with over 58,000 markings per inch, and thus go considerably beyond the usually accepted theoretical limits for its aperture. The recorded aperture of the objective, measured when purchased many years ago, was .57 N. A., and the markings of the diatom noted as varying from 57,000 per inch near the ends to 59,000 at centre of valve. After this resolution was found to be unmistakable, both measurements were repeated. The extreme rays entering the objective were determined by an Abbe apertometer placed on revolving stage with vernier and illuminated by edge of small lamp flame across the room. Two readings to tenth degrees at each "end reaction" agreed exactly, and the results, calculated by taking sine of the half angle multiplied by the refractive index of the apertometer, gave a numerical aperture of .588 which represents the outside limits.

A portion of the diatom valve, about half way between centre and end, which had been noted as being sharply resolved from raphe to margin, was then accurately counted under a high power, and the mean of a number of closely agreeing counts at slightly different points proved to be 58,800 per inch. The number in a full thousandth inch was counted to avoid the possibility of error in a smaller distance.

On glancing down the tube at back of objective when diatom was resolved, this result was at once accounted for, as only the blue and violet of the diffracted beam, with the merest tint of green, was admitted, and it therefore became apparent that for an objective as perfectly corrected as this one, we must use the column calculated for the "F" line, and not that for the "E" line, which is generally accepted as more nearly representing the effect of white light. It is hardly necessary to add that the resolution was effected with light from an ordinary microscope lamp, without color screen or other aid, as I never test an objective under any conditions, as to illumination or mounting medium, that will differ from those under which it will be used in routine work. It will be noted that the performance of this objective, when correctly interpreted, is strongly confirmatory of the Abbe theory.

Before proceeding to draw a conclusion from these heterogeneous and rambling notes, collated from records of work at odd times during past couple of years, I wish to reiterate the impartial attitude assumed in undertaking consideration of the subject. If there was any prejudice, it was in favor of at least a partial acceptance of the dioptric theory. When Wright's book was received and read, although recognizing many of its fallacies and rendered suspicious by its commendation of such old and discredited devices as the tandem microscope, better known here as "megamicroscope," and the insertion of a stop in the axis of the objective or Ramsden disk of the ocular to produce dark ground illumination, which equals the Abbe diffraction apparatus in its ability to conjure up "optical nightmares," I was nevertheless profoundly impressed, and after re-reading it several times and performing some of the experiments with which I had been previously unacquainted, concluded that the Abbe theory must undergo at least some modification; and if I no longer hold that view, it is principally as the result of experiments which were inaugurated with the idea of demonstrating exactly the reverse of the conclusions I was compelled to draw from them.

Much will of course depend on just what is understood to be included in Abbe's theory, on which various writers are by no means agreed. In his own papers Abbe appears to assume a thorough acquaintance with optical science on the part of his readers that few of us possess, hence he makes little reference to known facts and theories, but occasional passages show that he did not neglect to give them full consideration. In the start he unquestionably recognized the effect of absorption and refraction in producing the image, and his theory

requires the presence of both dioptric and diffracted beams. Furthermore, he lays stress on the fact that diffraction in the aperture of the objective will result in every point being imaged by a "dispersive circle" (the "antipoint" of Gordon) of greater or less size according to circumstances, and recommends the examination of a brightly illuminated object through a pinhole one-two hundred and fiftieth of an inch in diameter in a card or piece of tinfoil, to secure an idea of what must be the appearance of the best image that could be produced through a microscope magnifying five thousand diameters, even if the construction was perfect.

If his later application of the diffraction theory to the images of coarser details, earlier termed "absorption images," could be interpreted as a denial that any refracted rays, outside the dioptric beam, entered into the image at all, then indeed some modification is necessary, as most objects unquestionably refract light outside the dioptric beam; and not only do the simplest laws of refraction require that these rays find a place in the image, but there is no other way of accounting for what becomes of them. It is more likely, however, that Abbe regarded this fact as self-evident. At any rate, the important work he undertook was not to refute the dioptric theory, but to supplement it by accounting for phenomena which it then, as now, failed to explain. He has done this in so eminently satisfactory a manner that it would appear that his theory, in all essential details, must be accepted, unless someone can bring indisputable experimental proof of the formation of a microscopical image that goes beyond its possibilities. Any experiment that merely demonstrates failure to fully realize the possibilities of the Abbe theory, on account of interference with image formation due to diffracted rays arising in the aperture of objective, or anywhere except in the object itself, is not pertinent, as this unavoidable limitation was evidently fully recognized in evolving the theory.